REVIEW

Alkaloids in the human food chain – Natural occurrence and possible adverse effects

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Alkaloid-containing plants are an intrinsic part of the regular Western diet. The present paper summarizes the occurrence of alkaloids in the food chain, their mode of action and possible adverse effects including a safety assessment. Pyrrolizidine alkaloids are a reason for concern because of their bioactivation to reactive alkylating intermediates. Several quinolizidine alkaloids, β-carboline alkaloids, ergot alkaloids and steroid alkaloids are active without bioactivation and mostly act as neurotoxins. Regulatory agencies are aware of the risks and have taken or are considering appropriate regulatory actions for most alkaloids. These vary from setting limits for the presence of a compound in feed, foods and beverages, trying to define safe upper limits, advising on a strategy aiming at restrictions in use, informing the public to be cautious or taking specific plant varieties from the market. For some alkaloids known to be present in the modern food chain, e.g. piperine, nicotine, theobromine, theophylline and tropane alkaloids risks coming from the human food chain are considered to be low if not negligible. Remarkably, for many alkaloids that are known constituents of the modern food chain and of possible concern, tolerable daily intake values have so far not been defined.

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1 Introduction

Humans have used alkaloid-containing plants and animals since ancient times as poisons, stimulants, narcotics, insecticides, aphrodisiacs and medicines. In addition, alkaloid-containing plants have been and still are part of our regular diet. In a modern Western diet, they can be present as intrinsic constituents of, e.g. vegetables or tea, due to food processing, as food contaminants or as food flavourings. Consumers often consider botanicals and botanical preparations as safe because they are of the opinion that 'natural' equals 'safe'. For alkaloids, this is generally not the case and many are toxic at low doses.

Alkaloids are natural compounds with as most common characteristic a ring structure and a nitrogen atom. In most cases, the nitrogen is located inside the ring structure but there are exceptions to this rule [1]. More than 12 000 alkaloids have been described. To categorise the different alkaloids, in the present paper a classification is used based on the starting molecule in the biosynthetic pathway: ornithine, lysine, tyrosine, tryptophan or a non-amino acid precursor such as nicotinic acid, or purine analogues [2, 3].

The present paper focuses on the occurrence of alkaloids in the modern Western food chain and their possible adverse health effects. For each alkaloid group, after a short

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Abbreviations: ANZFA, Australia New Zealand Food Authority; **BfR,** German Federal Institute for Risk Assessment; **bw,** body weight; **COT,** Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (UK); **EAs,** ergot alkaloids; **EFSA,** European Food Safety Authority; **GAs,** glycoalkaloids; **JECFA,** Joint FAO/WHO Expert Committee on Food Additives; **MDI,** maximum daily intake; **MOS,** margin of safety; **NOAEL,** no observed adverse effect level; **PAs,** pyrrolizidine alkaloids; **QAs,** quinolizidine alkaloids; **TAs,** tropane alkaloids; **TDI,** tolerable daily intake; **VOD,** veno-occlusive disease

introduction, their occurrence in the food chain and concentrations in specific food items are presented, followed by a description of their toxicological effects including their mode of action and existing safety assessments as well as a conclusion about their current impact on food safety. Food-borne alkaloids included in the overview are alkaloids derived from ornithine (including pyrrolizidine alkaloids (PAs) and tropane alkaloids (TA)), from lysine (including piperidine alkaloids and quinolizidine alkaloids (QAs)), from tyrosine (isoquinoline alkaloids), from tryptophan (β-carboline alkaloids, quinoline alkaloids and ergot alkaloids (EAs)), from nicotinic acid (nicotine and myosmine), glycoalkaloids (GAs) also called steroid alkaloids and methylxanthine alkaloids derived from adenine/guanine.

2 Alkaloids derived from ornithine

Alkaloids derived from ornithine occurring in food and feed are the PAs and the TAs.

2.1 Pyrrolizidine alkaloids (PAs)

2.1.1 PAs: chemical characteristics and dietary occurrence

PAs occurring in food are mostly esters of 1-hydroxymethyl-1, 2-dehydropyrrolizidine, which may bear a hydroxyl group at position 7. Figure 1 presents the general structure of PAs as well as of some examples of PAs present in food. Over 500 PAs have been identified [4]. Food items that may contain PAs include grain-derived products, vegetables, honey, eggs, milk and offal [5]. In grain commodities, the PAs originate from contamination of the grains by seeds or plant fragments of PA-containing weeds growing in the crop (below 50 to over 6000 μg PAs/kg) [6].

The leaves of *Borago officinalis* (borage), used as spice and in salads and soups, have been found to contain levels of <10 mg of PAs/kg herb (http://www.itmonline.org/arts/pas.htm) [7]. The leaves or roots of comfrey (*Symphytum officinale*) may be used in herbal teas and remedies or as a vegetable (e.g. in salads), in spite of the fact that people are advised against this [4]. Comfrey contains several PAs, including echimidine (Fig. 1) and lasiocarpine [4]. The PA content of *S. officinale* leaves varies from 20 to 1800 mg PA/kg, and the roots of *S. officinale* contain 2500–2900 mg PA/kg [7, 8]. Moreover, blossoms and leaves of *S. vulgaris* have been detected in a salad mix (degree of contamination: 1.7%) and repeatedly in mixed salad and rocket salad in Germany [9–11]. Honeys made by bees foraging on *Senecio jacobaea* (tansy ragwort) may contain PAs including senecionine, jacobine (Fig. 1), seneciphylline, jaconine, jacoline and jacozine up to levels of 0.3–3.9 mg PAs/kg honey [12]. Honeys made by bees foraging mainly on *Echium* spp. contain as major alkaloid component echimidine (Fig. 1)

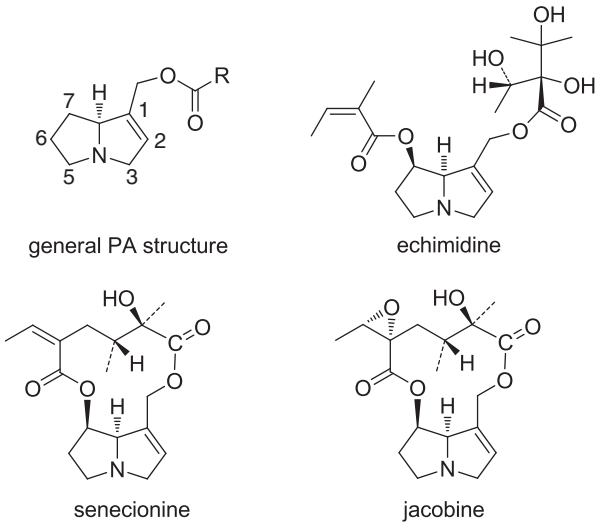


Figure 1. General structure and some representative PAs.

and minor quantities of other PAs at levels up to 0.54–1.9 mg PAs/kg of honey [13]. In Europe, honey originating from *Borago*, *Cynoglossum*, *Echium*, *Myosotis*, *Petasites*, *Senecio* and *Tussilago* species is of importance. Potential sources of honey in countries outside of the European Community are also *Ageratum*, *Chromolaena*, *Crotalaria*, *Eupatorium* and *Heliotropium* species [5]. Recent analysis of honeys available on the German/European market revealed that within the 216 samples analysed, 19 samples (9%) contained PAs, in the range of 0.019–0.120 mg/kg, calculated as retronecine equivalents [14]. A recent analysis of pure *Echium* honeys that were harvested in New Zealand in season 2006 revealed total PA contents mainly in the range of 0.3–0.4 mg/kg calculated in retronecine equivalents [15].

Another source of human exposure is milk from cows and goats, especially from those given feed contaminated with *S. jacobaea*. In an experimental study in which cows were administered dried *S. jacobaea* at levels up to 10 mg/kg body weight (bw)/day through a rumen canule, the milk contained up to 0.84 mg of alkaloids/L [4]. In human milk, PAs have been found during epidemics due to PA poisoning and, as a result, cases of veno-occlusive disease (VOD) have occurred in both neonates and small infants by this means [6]. Other dietary sources are eggs, which have occasionally been reported to contain PA levels from 5 to 168 μg PAs/kg [6].

2.1.2 PAs: possible adverse health effects and safety assessments

The acute and chronic liver toxicity of PAs is well known due to human and animal case reports and outbreaks of human poisonings by grain crops contaminated with seeds of PA-containing plants [4, 5]. Symptoms of acute PA poisoning are abdominal pain, ascites, nausea, vomiting, diarrhoea,

oedema and, very rare, jaundice and fever. This is associated with hepatic VOD involving obstruction of the small veins with sudden hepatomegaly (enlarged liver) and ascites, and may end with death [4, 7, 16]. A low long-term exposure to PAs through the intake of food yields chronic VOD leading to cirrhosis of the liver. Apart from the liver, other organs like the lungs (pulmonary hypertension) and the cardiovascular system (cardiac right ventricular hypertrophy) can be affected as well [4, 6, 7, 16]. The World Health Organization (WHO) suggested that the lowest intake of PAs that causes adverse effects in a human is 0.015 mg/kg bw/day, corresponding to 0.9 mg/day for a 60 kg person, based on the use of comfrey over a period of 4–6 months [4, 7]. PAs exert fetotoxic and teratogenic effects in higher doses [4, 8, 17].

Figure 2 gives an overview of the major metabolic pathways of PAs. Ester hydrolysis and *N*-oxidation represent detoxification processes [4, 16, 18, 19]. Bioactivation occurs via dehydrogenation of the pyrrolizidine nucleus to generate dehydro-alkaloids (pyrrolic derivatives), followed by acid catalysed cleavage of the C7-O bond, resulting in formation of a carbocation that may react with available nucleophiles like DNA, leading ultimately to liver necrosis and tumors [4, 16]. Several PAs and PA-containing plant materials have been evaluated by the IARC (International Agency for Research on Cancer) [20–22]. Lasiocarpine, riddelliine and monocrotaline have been classified by the IARC in group 2B (possibly carcinogenic to humans), and isatidine, retrorsine and senkirkine in group 3 (not classifiable) [20–22].

In 1992, the German Federal Health Office restricted the use of PA-containing plants in pharmaceuticals. For products with oral administration, exposure should not exceed 1 µg 1, 2-unsaturated PAs/day for a 6-wk period per year and they should not be used in pregnancy and during the lactation period. If a product is applied for more than 6 wk, the intake should not exceed 0.1 µg 1,2-unsaturated PAs/day [23].

For food in reference to the latter regulation, the German Federal Institute for Risk Assessment (BfR) recommends to minimise the exposure to PAs as far as possible [9, 24]. The BfR and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (UK) (COT) advice against the consumption of comfrey and products containing comfrey [9, 24, 25].

Regarding neoplastic lesions, the COT recommended to use a $BMDL_{10}$ (the lower confidence limit on the benchmark dose associated with a 10% response) of 0.073 mg/kg bw/day derived from a 2 year carcinogenicity study of lasiocarpine in male rats [26] to assess exposure for any PA. Allowing an MOE (margin of exposure) of at least 10 000, PA doses of up to 0.007 µg/kg bw/day were calculated to be unlikely to be of high priority for risk management. With respect to non-neoplastic effects, COT concurred with the Dutch National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) and concluded that by referring to the no observed adverse effect level (NOAEL) of 0.01 mg/kg bw/day in rats for hepatocyte cytomegaly observed in a rat National Toxicology Program study [27] and by applying an uncertainty factor of 100, a dose of 0.1 µg riddelliine/kg bw/day would not be expected to result in non-cancer effects. Regarding neoplastic effects based on the same study, RIVM established a virtual safe dose for PAs of 0.00043 µg/kg bw/day, leading to an increased risk of at most one person in a million developing cancer. This virtual safe dose is derived from the lowest dose leading to tumour development (hemangiosarcomas), which was 1 mg/kg bw/day [28].

The Australia New Zealand Food Authority (ANZFA) states that there is no evidence that PAs cause liver cancer in humans and suggests a provisional tolerable intake for PAs

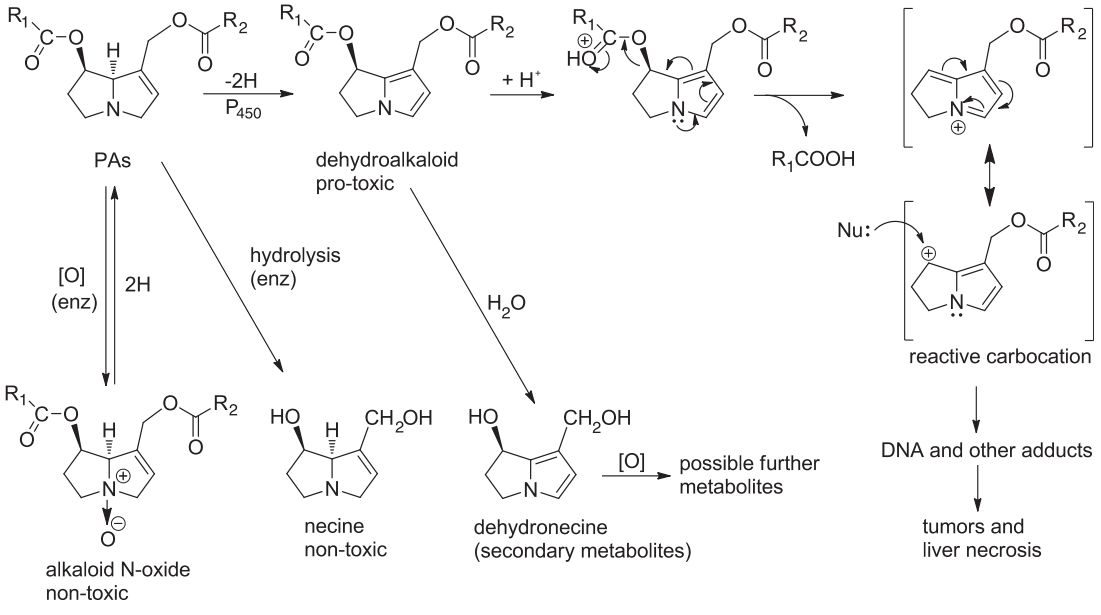


Figure 2. Metabolic pathways of PAs [4, 19].

of 1 μg/kg bw/day by considering a tentative NOAEL for VOD of 10 μg/kg bw in humans and applying an uncertainty factor of 10 [6].

The range of tolerable human intakes derived depends on the choice of endpoinds used and reflects the uncertainties due to lack of data for human risk assessment.

PAs have also been evaluated as undesirable substances in animal feed by the European Food Safety Authority (EFSA) stating that at present the data available for farm animal species do not allow tolerance levels to be set for individual PAs in feed material [29].

Some European countries have proposed maximum residue levels for PAs [30]. For example, in The Netherlands a maximum level of 0.1 μg PAs/100 g of food has been suggested [31]. Further details on existing regulations and recommendations are presented in a discussion paper published recently by the Codex Alimentarius Committee on Contaminants in Food [5]. In this report, management practices are recommended, such as measures for prevention of spreading of PA-containing plants, of contact of food producing animals with these plants and of contamination of food products (such as salads) with PAs [5].

2.1.3 PAs: conclusions

Dietary exposure to PAs may occur at levels that could be of toxicological concern, especially for subpopulations that consume relatively large amounts of specific PA-containing foods such as, for example, some types of honey. That is why at present regulatory agencies are focusing on better methods for detection of PAs in various foods and estimating the levels of exposure in preparation of future management measures.

2.2 Tropane alkaloids (TAs)

2.2.1 TAs: chemical characteristics and dietary occurrence

TA-containing plants are found in numerous families such as Solanaceae, Erythroxylaceae, Convolvulaceae, Brassicaceae and Euphorbiaceae [32]. There are more than 200 TAs and Fig. 3 presents the structure of some important TAs like (−)- and (+)-hyoscyamine, (a racemic mixture of the latter two alkaloids is known as atropine), (−)-scopolamine (also known as (−)-hyoscine) and cocaine. The alkaloid part is often hydroxylated and esterified with acids. TA-containing plants have been used medicinally and for folkloric purposes in many parts of the world. However, in the modern food chain, TAs occur mainly in feed, because seeds of especially *Datura* spp. plants containing high concentrations of hyoscyamine and scopolamine (total alkaloid content of seeds: 0.4–0.6%, from the alkaloids 55–74% amount to hyoscyamine and 15–20% amount to scopolamine [33]) can be

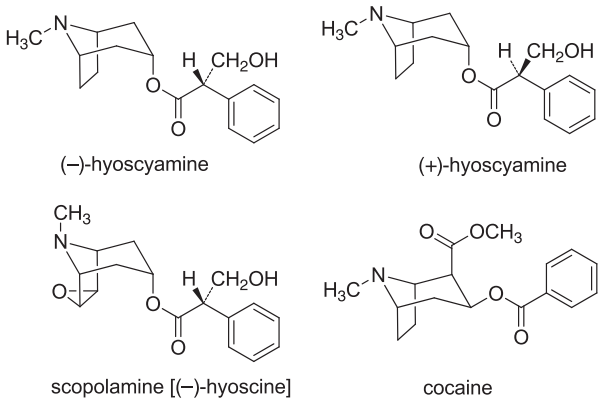


Figure 3. Structures of some representative TAs.

found as impurities in feed materials [32]. Contamination of feed with *Datura* seeds may occur especially in crops like soybeans and linseed [32]. In surveys in Germany, up to 31.1% of soybean and linseed products were contaminated with (parts of) *Datura* seeds and 65 of the 66 analysed samples contained scopolamine at levels between 0.1 and 33 mg/kg [34], although other studies reported soybeans to be much less contaminated [35]. Using different scenarios, EFSA estimated the intake by dairy cows to be 0.01–0.08 mg TAs/kg bw/day, by poultry 0.08–0.5.7 mg TAs/kg bw/day and by pigs 0.05–0.66 mg TAs/kg bw/day [32]. To estimate levels of TAs in food, information on transfer of TAs from feed to animal-based food products is required. Traces of scopolamine have been detected in eggs of 100 egg-laying hens given a mixture of scopolamine and hyoscyamine (98:2) at dose levels of 1.5, 15, 75 or 150 mg/kg feed for 3 months [36]. EFSA concluded that there is no information available on carry-over of TAs from feed into animal products under normal livestock conditions, except for the traces of alkaloids that have been found in eggs. No data are available on residues in milk or tissues from exposed animals. Furthermore, it was taken into consideration that certain animal species, such as poultry and rabbits, are considerably less sensitive to the exposure to TAs than others, presumably due to the expression of specific hydrolysing enzymes that inactivate the alkaloids [32].

A report by the RIKILT Institute of Food Safety concluded that carry-over does not appear to be a real problem [37]. The report also stated that based on the incidents with both animals and humans, it is to be expected that human foods potentially containing TAs would be herbal teas, herbal preparations (e.g. traditional Chinese or Ayurvedic), blueberries or black berries (either fresh of dried) and edible flowers. For blueberries, black berries and edible flowers exposure may occur when toxic plants (parts) are accidently mixed into edible plants during harvest or processing. The RIKILT report [37] indicated that recently this happened in France when *Datura* flower buds were mixed in with canned green beans, and that several cases of mistaken identity are reported where, for example, berries of deadly nightshade

resemble edible berries like blueberry. Contamination has also been found in buckwheat (for human consumption), soybean and linseed (animal feed). Based on this information, RIKILT concluded that these are the products that should primarily be monitored to prevent accidental exposure of humans to TAs. The incidence referred to for buckwheat [38] reports contamination of buckwheat flour with seeds from *Datura stramonium*. In September 2003, in Slovenia where buckwheat flour is commonly used in preparation of traditional dishes, cases of domestic food poisoning with a typical syndrome of TA toxicity were identified. All victims reported ingestion of a traditional dish made of buckwheat flour a few hours prior to the onset of symptoms. Examination of whole buckwheat grain showed up to 190 *D. stramonium* seeds/kg of grain.

RIKILT concluded that buckwheat-based products such as buckwheat-based flour, buckwheat groats grains, buckwheat kernels or roasted buckwheat grains also known as 'kasha' in the Eastern European cuisine are products that should be monitored to prevent accidental exposure of humans to TAs [37]. Intoxications of inhabitants of a Turkish village due to the consumption of bread made from flour estimated to contain almost 1% of seeds from *D. stramonium* have been reported in 1949 [39]. Furthermore, outbreaks of acute toxicity due to contamination of crops with 3–20% *Datura* seeds are reported from Ethiopia and Botswana [40, 41].

A special case of TAs in the diet may be cocaine, which is a constituent of coca leaves, extracts of which are used in cola drinks. A level of 0.4 μg cocaine/L has been reported for a coca leaf extract-containing soft drink (http://www.bfr.bund.de/cm/245/no_health_risk_from_the_cocaine_content_in_red_bull_simply_cola.pdf) [42].

2.2.2 TAs: possible adverse health effects and safety assessments

Several TAs are hallucinogenic and some are powerful anticholinergic drugs. Atropine, hyoscyamine and scopolamine are used therapeutically for different medical indications, their mechanism of action being based on their antagonistic action on muscarinic acetylcholine receptors [43]. The resulting effects are characterised by dryness of the mucosa of the upper digestive and respiratory tract, constipation, pupil dilatation and disturbance of vision, photophobia and dose-dependent occurrence of hyper- or hypotension, bradycardia or tachycardia as well as arrhythmias, nervousness, restlessness, irritability, disorientation, ataxia, seizures and respiratory depression [32]. Atropine administered orally in single doses of 0.5–1 mg up to three times daily is used in the treatment of smooth muscle spasms in the gastrointestinal tract, reported side effects being, e.g. slight cardiac slowing and dryness of mouth [43, 44]. Single oral doses in the range of 2–5 mg atropine are associated with rapid heart rate, dilated pupils, blurring of vision, difficulties of speaking and swallowing, dry and hot skin [32]. Oral doses of 10 mg and more may lead to rapid and weak pulse, ataxia, restlessness, excitement, hallucinations, delirium and coma [43]. Hyoscyamine is used in the treatment of visceral spasm in oral single doses of 0.15–0.3 mg up to four times daily, showing the same adverse effects as atropine (http://www.medicinescomplete.com/mc/martindale/current/) [45]. Scopolamine is administered orally in single doses of 0.15–0.3 mg up to four times daily in the prevention of postoperative dizziness and motion sickness. Typical adverse effects for anticholinergic drugs such as dryness of the mouth, changes in heart rate and disturbance of vision are reported within the range of the therapeutical dosage [45, 46]. If no degradation due to baking would occur, consumption of 200 g of bread made from flour contaminated with 0.1% of *Stramonium* seeds containing 0.5% of alkaloids would be roughly equivalent to a single dose of 1 mg of atropine.

Intoxications by TAs reported for children, teenagers and adults result mainly from abuse (because of the hallucinogenic effects), experimentation with TA-containing plants or accidental exposure [32, 37, 47, 48].

Cocaine is a drug of abuse in many countries. It has local anaesthetic properties but its therapeutic use nowadays is very limited [45]. The BfR presented a health risk assessment of the cocaine content of a coca leaf extract-containing soft drink and concluded that no health risk is to be expected from consumption of this product because of its low cocaine content. According to information in the scientific literature, the lowest dose that may lead to an adverse effect is a daily intake of 4800 μg cocaine per person. Assuming a high daily consumption of 1.7 L, the margin of safety (MOS) between the consumed amount of cocaine and the amount upwards of which adverse effects may occur is a factor of approximately 7000 [42].

2.2.3 TAs: conclusions

Following absorption, hydrolysis by specific hydrolytic enzymes inactivates TAs, and this may limit their toxicity in certain animal species [32]. Carry-over to animal products in the food chain appears to be limited and in general levels of TAs in food and beverages seems to be low nowadays. Overall, it can be concluded that under production conditions applying to modern standards, it is unlikely that residues of TAs in food and beverages constitute a health risk for consumers. However, the public should be well informed about risks of abuse and accidental poisoning regarding TA-containing plants.

3 Alkaloids derived from lysine

Alkaloids derived from lysine are piperidine, QAs and indolizidine alkaloids but only the first two groups occur in the Western diet and are discussed below.

3.1 Piperidine alkaloids

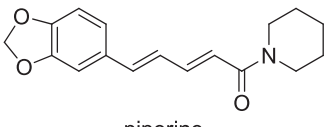
3.1.1 Piperidine alkaloids: chemical characteristics and dietary occurrence

The piperidine alkaloid piperine (Fig. 4) is responsible for the biting, pungent taste of peppers and is used as a spice [49]. The main human dietary exposure comes from this source. Another possible source is brandy to which pepper is added to impart a pungent taste. Black pepper (*Piper nigrum*) contains 5–9% (50–90 g/kg) piperine [50]. A daily consumption of 0.33 g of black pepper by a 60 kg person [51] would result in an intake of 16.5–29.7 mg piperine/person/day.

3.1.2 Piperidine alkaloids: possible adverse health effects and safety assessments

Black pepper might be a cause for gastric mucosal injury especially after chronic consumption. Myers et al. [52] assessed the effects of black pepper on the gastric mucosa using double-blind intragastric administration of the spice (1.5 g) to healthy human volunteers, with aspirin (655 mg) as positive control. Severe bleeding was observed in one subject after intake of black pepper. Several cases of fatal pepper administration have been reported in the literature [53, 54]. Mechanical obstruction and mucosal oedema were identified as mechanisms of death [54]. However, the observed effects cannot clearly be linked to piperine since piperine was not administered as such, and fatalities/severe side effects might have been caused by mechanical means because exposure was via (accidental) aspiration.

According to Jellin et al. (2007) black and white peppers when used orally in large amounts are regarded as likely unsafe for children and pregnant women. There are insufficient data on possible abortifacient effects in large amounts [50]. It is important to stress that these black and white peppers are not related to the so-called chili peppers, which are plants belonging to the genus *Capsicum* and known to contain capsaicin and other capsaicinoids. These capsaicinoids are also an irritant for mammals, including humans, and produce a sensation of burning in any tissue with which they come into contact [55]. Capsaicin, which originates from phenylalanine and bears an exocyclic nitrogen atom here, is regarded as a non-alkaloid derivative of an amino acid and not included in this review. Piperine has also been found to inhibit human CYP3A4 and *P*-glycoprotein in vitro, proteins that are important for the metabolism and transport of xenobiotics and their metabolites [56]. By inhibiting drug metabolism, piperine may increase the bioavailability of other compounds [57, 58].



piperine

Figure 4. Structure of piperine, a piperidine alkaloid.

3.1.3 Piperine: conclusions

When used orally in amounts commonly found in foods, piperine-containing white and black peppers do not represent risks for human health. However, when consumed at much higher doses, these peppers might induce unfavourable effects including gastric mucosal injury, and extreme consumers of black pepper may be at increased risk. Due to its effects on drug metabolism, piperine-containing spices should be taken cautiously by individuals taking other medications.

3.2 Quinolizidine alkaloids (QAs)

3.2.1 QAs: chemical characteristics and dietary occurrence

The most common QAs are sparteine and lupanine, α-isolupanine, 13α-hydroxylupanine and anagyrine (Fig. 5). Anagyrine is not found in cultivars for human use and plant-breeding programmes ensure this remains the case [59, 60].

European lupins or 'bitter lupins', which are predominantly consumed in Southern Europe as seeds (beans), have a high alkaloid content of 1–2% (10–20 g/kg). The alkaloid level can be reduced through a de-bittering process involving soaking or washing with water. The level of alkaloids in de-bittered lupins is approximately 500 mg/kg (0.5 g/kg; 0.05%) [59]. Sparteine is present especially in European varieties whereas lupanine is typical for Australian species. The Australian varieties developed by

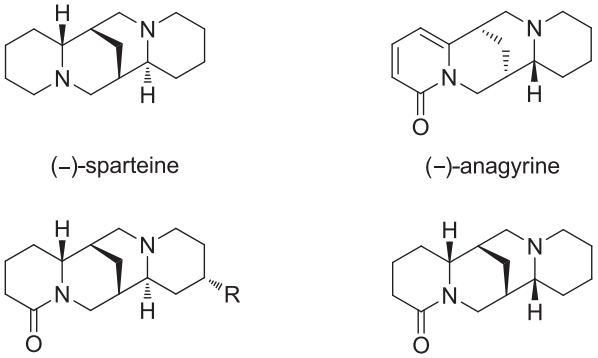


Figure 5. Structures of some representative QAs.

plant-breeding programmes are called 'sweet lupins' as they contain a strongly reduced amount of total alkaloids. The mean total alkaloid content of the marketed Australian sweet lupin (*L. angustifolius*) seed is on average 130–150 mg/kg (0.13–0.15 g/kg; < 0.02%) of which 70% is lupanine [59].

The major source of QA exposure is lupin flour from low alkaloid varieties used to substitute for a small percentage of wheat flour. Lupin flour is also used to replace soybean flour. It is further used in food formulations to replace soy flour in food commodities and also in lupin-based meals, pastas, pastries, cakes, biscuits, snacks, tempe, bread, miso, soy sauce, dairy/tofu product- and coffee substitutes [59, 60]. For example, a coffee surrogate, made of roasted lupin beans, has an alkaloid content that almost reaches the Australian limit for finished food products of 200 mg alkaloids/kg of product [60].

3.2.2 QAs: possible adverse health effects and safety assessments

Sparteine and lupanine both display moderate acute toxicity, the former being the more toxic one. The acute oral LD_{50} (lethal dose for 50% of the population) values in rats for lupanine is 1664 mg/kg bw [61]. There are some cases of acute toxicity in humans who ate lupin beans, which had not been previously de-bittered. Persons suffered from blurry vision, dry mouth, facial flushing and confusion [62, 63]. Marquez et al. reported a single case of a young man who drank 0.5 L of water that had been used for the de-bittering of lupin seeds [64]. He suffered from sudden weakness, palpitations, extrasystoles and different anticholinergic symptoms. In another study, accidental ingestion of unripe lupin seeds resulted in nausea, migraine, abdominal pain, bradycardia and respiratory depression [65]. QAs exert a blocking effect on the nicotinic cholinergic receptor and they are weak antagonists at the muscarinic cholinergic receptor [65]. Neurological (weakness, dizziness, mydriasis, anxiety, confusion, malaise, loss of coordination, visual disturbances and dry mouth), cardiovascular (dysryhthmias) and gastrointestinal (nausea, vomiting) symptoms are due to these anticholinergic effects.

In a 90-day feeding study in which rats were given diets supplemented with 20% lupin protein from the sweet lupines *L. albus* and *L. luteus*, containing 0.05% (0.5 g/kg) and 0.09% (0.9 g/kg) lupin alkaloids, no adverse effects were observed in terms of food intake, organ weight and microscopic examinations [66]. The actual levels of alkaloids present in the diets were not reported, but taking into account the protein levels in the sweet lupines of 35 and 39% and assuming full incorporation of the alkaloids into the diet, the lupin flour dietary levels would amount to about 290 and 460 mg alkaloids/kg diet, respectively, resulting in dietary intakes of about 14.5 and 23 mg lupin alkaloids/kg bw/day.

In a 9-month two-generation study, rats were fed with a diet enriched with lupin flour from a newly developed cultivar *L. albus* cv. *Multolupa* containing 250 mg lupanine/kg diet [67]. No adverse effects were observed on fertility or lactation or any other parameter investigated [67]. This dietary level was estimated to amount to approximately 25 mg lupanine/kg bw/day for the young rats and 12.5 mg lupanine/kg bw/day for the older rats.

In another 90-day study, the dietary concentration was 50 mg of lupin alkaloids/kg diet for the control group and 250, 1050 and 5050 mg of alkaloids/kg diet for the treated groups, resulting in intakes of at most 6.6, 31.9, 128.9 and 597.1 mg of alkaloids/kg bw/day for the male and 6.5, 33.4, 135.3 and 611.1 mg of alkaloids/kg bw/day for the female rats in the first days of the study. An increase in hepatic foci (5/50) in the top-dose females appeared to be the major dose-dependent adverse effect [61].

We concluded that together these subchronic toxicity studies point at a lowest NOAEL for lupin alkaloids in rats of at least 12.5 mg lupin alkaloids/kg bw/day.

Petterson et al. concluded that clinical toxicity is unlikely to result from the use of lupin seed in foodstuffs [65]. The ANZFA concluded that traditional use of lupin seeds in Europe suggests that a daily dose of 0.35 mg lupin alkaloids/kg bw (~20 mg lupin alkaloids/day) can be tolerated in human adults without adverse effects and that if an uncertainty factor of 10 is applied to account for the uncertainties in the data and particularly to take into account likely human variation, the provisional tolerable daily intake for humans is 0.035 mg lupin alkaloids/kg bw/day [59].

3.2.3 QAs: conclusions

Several case reports on adverse effects of QAs upon accidental intake of lupin-containing foods have been reported. These case studies resulted especially from intake of diets containing lupin flour. Effects on human health reflecting neurological, cardiovascular or gastrointestinal symptoms can be observed either if lupin seeds are eaten unripe or non-de-bittered. Tolerable daily intake (TDI) values for QAs have not been established, although taking the NOAEL of 12.5 mg/kg bw/day and a MOS of 100, one may conclude that a daily intake of 0.125 mg lupin alkaloids/kg bw/day would not be of safety concern. This value is in the same range as the provisional tolerable daily intake of 0.035 mg lupin alkaloids/kg bw/day derived by the ANZFA based on traditional use of lupin seeds. This would imply that for a 60 kg person, a daily intake of 2.1–7.5 mg alkaloids can be tolerated. Given that the seeds of modern cultivars (i.e. 'sweet lupins' of *L. angustifolius*) contain < 200 mg alkaloids/kg, this would imply a daily consumption of at most 10.7–37.2 g of sweet lupin seeds. This estimate suggests that good quality control of lupin-containing foods is necessary.

4 Alkaloids derived from tyrosine

Isoquinoline alkaloids are derived from tyrosine. Morphine is the most well-known example occurring in the Western diet and is discussed below.

4.1 Isoquinoline alkaloids (morphine)

4.1.1 Isoquinoline alkaloids (morphine): chemical characteristics and dietary occurrence

Isoquinoline alkaloids occur in the latex of *Papaver somniferum* (opium poppy). The latex of the immature capsules, which is released by incisions and which has dried on the capsule surface, is called opium. It contains approximately 20–25% (200–250 g/kg) alkaloids. So far, around 50 different alkaloids have been isolated from opium. The main alkaloids of opium are morphine (Fig. 6), which is present in the largest concentration (depending on origin 7–20%), codeine (0.3–6%) and thebaine (0.2–1%) [68, 69]. The seeds are the only part of the plant used as food and their alkaloid content varies over wide ranges. Morphine (<0.1–620 mg/kg) and codeine (0.1–57 mg/kg) are usually detected in the seeds probably due to contamination with the latex or other parts of the plants [70–72]. The differences in the alkaloid content in the seeds depend on the geographical origin, soil type, climate, year of harvest and the cultivar as well as seed treatments [73]. Washing the seeds prior to use removes as much as 45.6% of free morphine [73]. The initial morphine concentration was reported to be reduced by 10–50% by baking and by 98–100% by washing combined with subsequent heating [74].

The small black, brown, white or blue seeds that are a common garnish on bagels, rolls, muffins, breads are consumed in some countries in larger amounts in the form of cakes and desserts, and are also used to produce an edible oil and in making jams [71].

4.1.2 Isoquinoline alkaloids (morphine): possible adverse health effects and safety assessments

Morphine acts by binding to opioid receptors in the central and peripheral nervous system and the gastrointestinal tract. Activation of the opioid receptors in these organs mediates both the beneficial as well as the adverse effects of opium alkaloids.

Acute morphine intoxication normally manifests itself by the three symptoms miosis, respiratory depression and unconsciousness (coma). Respiratory depression is the most important risk after an opioid overdose. The direct cause of death is respiratory arrest [75, 76]. In individuals who do not show any tolerance development, serious toxic symptoms may already occur after the oral administration of 40–60 mg of morphine [76]. For adults, doses of 200 mg of morphine may be acutely lethal [77]. Other sources give a range of 230–1140 mg of morphine for the oral lethal doses in the case of non-opiate-dependent adults whereas babies and infants are far more sensitive [69].

Long-term use can lead to tolerance development as well as psychological and physical dependence. Individual sensitivity varies markedly. This applies both to the desired effects and adverse reactions in medicinal usage [75, 76, 78]. In animal experiments, morphine had a negative impact on development and reproduction [79–82]. It has been reported that the consumption of commercially available poppy seeds, e.g. in the form of desserts with approximately 10–20% poppy seeds or poppy seed cakes, can lead to light-headedness and enteroparesis in sensitive individuals [83].

The symptoms described in a consumer complaint made to the official German food control also agree with the range of toxicological actions of morphine. After eating a dish, which had been sprinkled with a mixture of ground poppy seed and sugar, a consumer observed an 'uneasy feeling' in her head, had to vomit and felt like she had a hangover the next day. The person concerned had ingested approximately 75 g of blue poppy seeds, containing 210 mg morphine/kg and 39 mg codeine/kg. This corresponds to an intake of 16 mg of morphine and 3 mg of codeine. The poppy seeds were deemed to have a health-injuring potential within the intendment of Article 14 para 2a and para 4 of Regulation (EC) 178/2002 [72, 84].

Moreover, there is a report of a poisoning case with severe health impairment of a 6-wk-old female infant who received a boiled poppy seed preparation by her mother to induce sleeping. The infant had been given 75 mL of strained milk from a mixture of 200 g poppy seeds boiled in

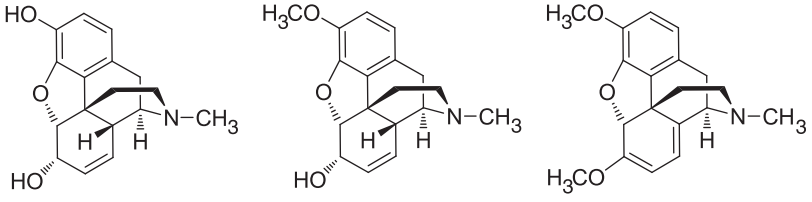


Figure 6. Structures of morphine, codeine and thebaine.

500 mL milk and developed respiratory depression culminating in respiratory arrest. Toxicological examinations revealed that the morphine level in the serum of the infant was still as high as 4.3 μg/L the following day. The infant had to be respirated and administered an antidote. After 10 days, she could be discharged from hospital in a healthy condition [85].

BfR recommends a 'provisional daily upper intake level' for morphine of 6.3 μg/kg bw/day. This value indicates the morphine intake that should not be exceeded during one meal or several meals distributed over the day. The calculation was based on the lowest effective single oral therapeutical dose of morphine being 1.9 mg (equivalent to 31.7 μg/kg bw at a body weight of 60 kg) and applying a fivefold uncertainty factor [10, 72]. This factor takes into account the existing uncertainty concerning the threshold doses of health-relevant effects (in particular psychomotor effects), possible interactions (e.g. with other opium alkaloids in poppy seeds, central nervous pharmaceuticals and alcohol) and inter-individual variations in sensitivity [72]. Considering a high level of consumption (a meal containing 100 g poppy seeds), a provisional guidance value for morphine in poppy seeds of 4 mg/kg was derived [72]. For poppy seeds only used in maximum daily portions of 20 g, a maximum level for morphine of 20 mg/kg was recommended [72].

4.1.3 Isoquinoline alkaloids (morphine): conclusions

Manufacturers and consumers should be advised to use treatment methods (e.g. washing, heating, baking) to reduce the contamination of poppy seeds with opium alkaloids. In the case of ingestion of small amounts of poppy seeds as used for decoration of pastries, the effects of morphine on human health seem to be negligible.

5 Alkaloids derived from tryptophan

Of the large group of the indole alkaloids derived from tryptophan, congeners of three subgroups occur in the Western diet. These three groups are: simple indole (carboline) alkaloids, quinoline alkaloids and EAs and these are discussed in more detail below.

5.1 Simple indole (carboline) alkaloids: β-carboline alkaloids

5.1.1 β-Carboline alkaloids: chemical characteristics and dietary occurrence

Tetrahydro-β-carbolines or 'tryptolines' and β-carbolines are a group of alkaloids possessing a tricyclic pyrido[3,4-*b*]indole ring with various substituents at C-1, C-3 and C-7 (Fig. 7) [2]. They occur in various plant families like Apocynaceae, Bignoniaceae, Elaeagnaceae, Gramineae, Leguminosae, Passifloraceae, Rubiaceae and Zygophyllaceae, fungi, processed food, marine organisms, insects, mammals, human tissues and fluids [86, 87]. Some examples of β-carbolines are harman, norharman, harmine and harmol (Fig. 7). Examples of 3,4-dihydro-β-carbolines include harmaline and harmalol. An example of a tetrahydro-β-carboline is eleagnine (Fig. 7).

In addition to their natural occurrence, β-carbolines are also formed during cooking, food production, processing and storage [88]. Many factors, both chemical and technological, influence the formation of β-carbolines including pH, storage time, temperature, precursors, preservatives, antioxidants, oxidants, yeasts, processing conditions (fermentation, smoking, cooking, heating) [89, 90]. For example, grilling of meat on an open flame results in high levels of both harman and norharman [90]. Additionally, β-carbolines are found in grill scrapings and pan residues, in vegetable-derived flavours, meat extracts and bouillon cubes [90]. Pretreatments for preserving foods (e.g. freezing, steaming) may lead to more β-carboline formation during further preparations [91, 92]. The presence of β-carbolines in various food items including cooked fish, cooked meat, bread, breakfast cereals, soups, alcoholic beverages, vinegar, soy and Tabasco sauce, brewed coffee, fruit juices and jams [90, 93] results in detectable levels of β-carbolines like harman and norharman in human biological tissues and fluids [90, 94]. In the human body, β-carbolines are also formed endogenously [95] but compared to exposure to

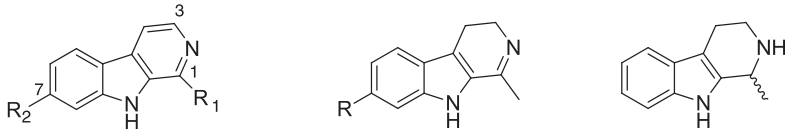


Figure 7. Structures of some representative β-carbolines [86].

exogenously formed β-carbolines via the diet, endogenous formation is negligible [90].

One of the major dietary exogenous sources of harman and norharman is brewed coffee. A standard cup of coffee, made of 170 mL of water and 7 g of coffee, contains up to 24 μg of β-carbolines/cup [94]. Ground instant and decaffeinated coffee contain smaller amounts. Maximum daily intake (MDI) of the main β-carbolines has been estimated to be 4.1 μg/kg bw (norharman) and 1 μg/kg bw (harman) [90].

5.1.2 β-Carboline alkaloids: possible adverse health effects and safety assessments

β-Carbolines are known to be neurotoxic, inhibit cyclin-dependent kinases, topoisomerase and monoamine oxidase, and to interact with benzodiazepine receptors and 5-hydroxyserotonine receptors [87, 96, 97]. Furthermore, these chemicals also demonstrated a broad spectrum of pharmacological properties including sedative, anxiolytic, hypnotic, anticonvulsant, antitumour, antiviral, antiparasitic as well as antimicrobial activities [87]. The physiological role of β-carbolines has yet to be shown. They may act as neuromodulators. It has been suggested that some β-carbolines act as the physiological ligands (agonists) of the benzodiazepine receptors, but the physiological β-carbolines so far known seem to have other effects, such as the inhibition of monoamine oxidase-A or 5-hydroxyserotonine uptake in low concentrations [96, 97].

β-Carbolines are also of concern because they are known to intercalate with DNA. Harman and harmine were shown to be genotoxic in V79 Chinese hamster lung fibroblasts in vitro using the Comet assay and also in a chromosome aberration test [98].

Because of structural similarity to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), carbolines and/or *N*-methylated carbolinium ions have been proposed as putative neurotoxins and initiators of Parkinson's disease, but this remains to be established [99–101].

5.1.3 β-Carboline alkaloids: conclusions

β-Carbolines are unavoidable components of the Western diet and the increased consumption of meat, especially cooked at high temperatures (> 200°C) has raised a concern given the neurotoxicity and genotoxicity of these compounds. Coffee, cooked meat and fish, cooked vegetables, citrus juices and especially for small children purees are mainly responsible for the daily exposure to β-carbolines. So far, safe exposure levels for these unavoidable contaminants in the food chain could not be defined. Additional information is needed, for example, with respect to the relative contribution of exogenous exposure compared to endogenous formation of these β-carbolines and regarding their possible carcinogenic potential.

5.2 Quinoline alkaloids (quinine)

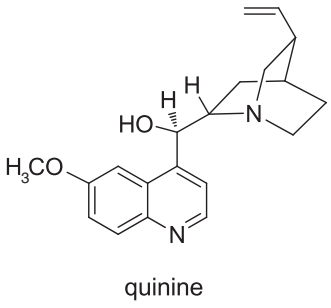
5.2.1 Quinoline alkaloids (quinine): chemical characteristics and dietary occurrence

The best-known quinoline alkaloid is quinine (Fig. 8). Quinine occurrs in considerable amounts in the bark of Cinchona species, e.g. *C. pubescens* and *C. officinalis*, from which it is isolated for pharmaceutical and food uses [102]. It is applied to treat malaria (650 mg of quinine sulfate every 8 h for 3–7 days) and nocturnal leg cramps (200–400 mg of quinine sulfate dihydrate/day for a maximum of 5 wk) [76, 103].

The main dietary exposure to quinoline alkaloids is through beverages. Quinine is a flavour component of tonic water, bitter lemon and vermouth because of its pleasant bitter taste. In some countries like the USA and Germany, non-medical use of quinine is regulated. The latest version of the German Flavourings Ordinance of May 2, 2006 (Annexes 4 and 5) gives the following maximum levels in drinks, calculated as quinine: total 300 mg/kg in spirits and 85 mg/kg in non-alcoholic beverages [104]. In the USA, the quantity in soft drinks is limited to 83 mg/L [105].

5.2.2 Quinoline alkaloids (quinine): possible adverse health effects and safety assessments

Quinine exerts effects on skeletal muscles, which have clinical implications. It increases the tension response to a single maximal stimulus delivered to muscle directly or through nerves, but it also increases the refractory period of muscle. The excitability of the motor endplate region decreases so that responses to repetitive nerve stimulation and to acetylcholine are reduced. It may produce alarming respiratory distress and dysphagia in patients with myasthenia gravis [76]. Because of easy placental accessibility, oxytocic action and embryotoxicity at high doses, pregnancy is a contraindication as are hypersensitivity to Cinchona alkaloids, bradycardia and other cardiac dysrhythmias of clinical relevance, tinnitus, prior damage to the optic nerve, glucose-6-phosphate-dehydrogenase deficiency (symptom: haemolytic anaemia) and myasthenia gravis. Quinine may



quinine

Figure 8. Structure of quinine.

enhance the effects of cardiac glycosides, muscle relaxants and anticoagulants [76, 103].

Consumption of tonic water containing up to 120 mg quinine hydrochloride by healthy volunteers, aged 18–53 years, daily for 14 days did not cause any adverse effects [106]. The plasma quinine level of 1–2 mg/L associated with this consumption is well below the threshold level of 10–15 mg/L associated with visual disturbances [107], hypoglycaemia [108] or acute poisoning [109, 110].

One study [111] reported about a neonate, which was described as jittery 24 h after birth. From the 24th week of pregnancy until birth in the 41st week of pregnancy, the mother had drunk 1.1 L of tonic water daily, equivalent to a daily quinine intake of 60 mg. The authors suggested that quinine withdrawal caused the infant's symptoms. Severe reactions, e.g. thrombocytopenic purpura indicated as 'bitter lemon purpura' or haemolytic anaemias (symptom: petechiae), in some cases complicated by kidney failure or disseminated intravasal coagulations, have been described after consumption of quinine-containing beverages [112–115]. Quinine intake from larger amounts of tonic water (1–2 L/day) may lead to the need to readjust anticoagulant treatment [116].

The JECFA (Joint FAO (Food and Agriculture Organization of the United Nations)/WHO Expert Committee on Food Additives) re-assessed quinine in 1993 and concluded that current levels of up to 100 mg/L (as quinine base) in soft drinks were not of toxicological concern. The contribution of other uses of quinine in food and alcoholic beverages relative to the total daily intake was considered to be negligible. The Committee also noted that certain consumers showed idiosyncratic hyper-reactivity to quinine, and reiterated its recommendation that the consumer should be informed by appropriate means of the presence of quinine in beverages [117]. In agreement with this, according to Directive 2002/67/EC of the European Commission of 18 July 2002, quinine used as a flavouring in the production or preparation of a foodstuff must be mentioned by name in the list of ingredients indicated in Article 3(1)(2), of Directive 2000/13/EC, immediately after the term 'flavouring'.

Based on the data available and for the purposes of preventive health protection, the BfR advises against consuming quinine-containing beverages during pregnancy [118]. BfR also recommends that people who are advised by their doctors against taking quinine based on existing contraindications should also refrain from consuming quinine-containing drinks. Patients with cardiac arrhythmia and people who take medications that interact with quinine should only drink quinine-containing drinks after consulting their doctors. This applies in particular to medications that inhibit blood coagulation [10, 118]. In view of warnings having been issued about persons who may be particularly sensitive to quinine, EFSA recommended that the toxicological database on quinine should be reconsidered [119].

5.2.3 Quinoline alkaloids (quinine): conclusions

Quinine consumption especially in larger amounts may cause health problems for certain susceptible consumer groups. Normal domestic consumption of quinine-containing drinks is unlikely to cause adverse effects on human health [106]. However, to be on the safe side, pregnant women should be advised against quinine-containing drinks. In addition, people who are advised against taking quinine as a medication based on existing contraindications and patients who take medicines that interact with quinine, especially medications that inhibit blood coagulation, should refrain from consuming quinine-containing drinks or consult their doctor [10, 118]. A TDI for quinine is not available.

5.3 Ergot alkaloids (EAs)

5.3.1 EAs: chemical characteristics and dietary occurrence

The third group of tryptophan-derived alkaloids occurring in the Western diet are the EAs. Ergot is the dried sclerotium of the fungus species *Claviceps* from which those of *purpurea* are the most important in Europe and are developing mainly on rye, *Secale cereale*. The ergot sclerotia contain 0.15–0.5% (1.5–5g/kg) alkaloids, and more than 50 different EAs have been characterised [2, 120]. EAs are derivatives of lysergic acid, which are mainly encountered as amides with a water-soluble amino alcohol moiety (up to ~20% of the total alkaloids) as in ergometrine (Fig. 9) or as water-insoluble tripeptides (up to ~80% of the total alkaloids) as in ergotamine (Fig. 9) [120].

Ergots may be harvested together with grain and contaminate flour or animal feed. EAs in wheat flour range from 4 to 100 µg/kg and in rye flours from 15 to 400 µg/kg. Exceptionally, rye flours may contain EAs in concentrations as high as 2.3–7.3 mg/kg [121, 122]. Separation of the sclerotia from grain, or the use of fungicides during cultivation of the crop, removes most of the EAs [2]. The few data available, however, do not provide any evidence that EAs

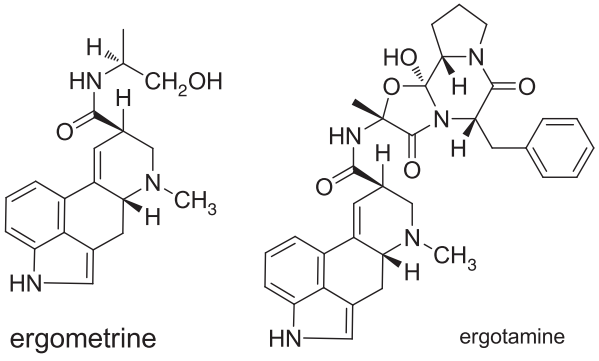


Figure 9. Structures of ergometrine and ergotamine.

accumulate in edible tissues, including milk and eggs and thus food from animal origin is unlikely to be an important source of human exposure [123].

5.3.2 EAs: possible adverse health effects and safety assessments

EAs are mycotoxins that interact agonistically with monoamine receptors such as dopamine receptors and adversely affect the cardiovascular, nervous, reproductive and immune system of humans and animals [124–132]. Reports on EA-induced adverse effects include safety studies in animal models with selected EAs [127–130], a report on field outbreaks of EA toxicity in cattle resulting in loss of milk production, loss of body mass and reduced fertility [131] as well as a human intoxication reporting myocardial infarction upon prolonged therapeutic use of ergotamine [132]. Pharmaceutically, ergotamine tartrate is used in the treatment of migraine. The usual oral dose is 1–2 mg and not more than 6 mg should be given per 24 h. The total weekly dose is limited to a maximum of 12 mg [133]. Salts of ergometrine are used in the active management of the third stage of labour, and to treat postpartum haemorrhage caused by uterine atony. When applied orally, the dosage amounts to 0.2–0.4 mg two to four times daily [133].

EAs in food may result in adverse effects as illustrated by a recent case of poisoning with buckwheat [134]. Chronic consumption of rye infected by EAs has resulted in the disease ergotism. Ergotism occurred during the Middle Ages in Europe, also known as St. Anthony's fire. Ergotism exists in two forms, convulsive (neurologic) and gangrenous (vasoconstrictor). Convulsive symptoms are weariness, giddiness, dimness of sight, loss of sensibility, voracious appetite, convulsions, involuntary muscle contractions, painful flexion of joints, tingling of the skin, headaches, fever, hallucinations, mania and delirium followed by death. Gangrenous ergotism starts with tingling effects in the fingers and toes, is followed by dry gangrene of toes and limbs and may finally lead to loss of limbs. It results also in experienced formication, voracious appetite and the extreme vasoconstrictive properties of ergotamine cause leg ischaemia [120]. Case studies investigating the use of ergotamine tartrate (10 mg or more/week, for 6 months) in alleviating migraine have been performed. About half of the patients showed symptoms of ergotism [135, 136]. Typical adverse effects known from the medical use of ergotamine and ergometrine salts include symptoms of ergotism [133].

Although the exact mechanism of action at the receptor level remains to be elucidated, most of the (adverse) effects of EAs can be associated with an agonistic action on α-adrenergic, dopaminergic D_2 and serotonergic (in particular $5HT_{1B/1D}$) receptors [123, 133]. For example, the subacute toxicity of the EA -ergocryptine has been studied in rats and has been ascribed to interactions with the central dopaminergic system with a NOAEL of 4 mg/kg diet, amounting to about 0.2 mg/kg bw/day [127, 128]. In another study, the subacute toxicity of ergometrine maleate was characterised in rats pointing at a NOAEL of 10 mg ergometrine maleate/kg diet equivalent to about 1 mg ergometrine maleate/kg bw/day corresponding to 0.74 mg ergometrine/kg bw/day [129]. The authors of this study also indicated that the maximum concentration of EAs in rey, wheat and oats in The Netherlands was reported to amount to 2.36 mg/kg. Assuming an average human daily consumption of cereal products of 200 g per person, this would result in a MDI of 7.8 μg EAs/kg bw/day. Compared to the NOAEL from the subacute toxicity study, this would result in a MOS of about 100.

Ergotamine has been suspected of being teratogenic [137] and data from animal studies have shown maternal toxicity. In pregnant women, ergotamine, prescribed against migraine, has led to low birthweights of newborns and to pre-term births. These associations might be explained by an effect of ergotamine on the placenta and increased uterine contractivity [138, 139].

In the European Union, there is a legal limit of 1000 mg of ergot sclerotia/kg of animal feed containing unground cereals according to Directive 2002/32/EC [140]. Daily intake of EAs from cereals by the average Swiss consumer is about 5 μg [141]. Investigations in Germany indicated an increase in the occurrence of *Claviceps purpurea* infections over the last 10 years [123]. Data on dose-related pharmacological and toxicological effects are available due to the medical use of ergotamine and ergometrine and have been used as a basis for the risk assessment of ergot contamination of rye flours. The BfR reported that levels of total EAs in four out of five rye flour samples analysed in 2004 varied between 2308 and 3138 μg/kg flour. In one sample the concentration of total EA amounted to 7255 μg/kg flour. Daily intake of 250 g of this rye flour was estimated to result in intake of 834 μg ergotamine and 91 μg ergometrine/day. For ergotamine this intake was reported by the BfR to reach the lowest therapeutic daily dose of 1 mg and to exceed the proposed maximum level for month-long intake of 0.67 mg/day. Based on these considerations, the BfR concluded that intake levels of ergotamine and ergometrine with contaminated flour in the range of the therapeutic dosage or with an insufficient MOS compared to the therapeutic dosage are considered to present a health risk [121, 122]. The U.S. Department of Agriculture grains division has set a tolerance limit of 0.3% of sclerotia (by weight) (equal to 3 g/kg) for grain in commercial trade [142].

5.3.3 EAs: conclusions

Contamination of foods with EAs is a matter of concern also for the modern food chain. Occurrence of *C. purpurea* infections has shown a tendency to increase in recent years. The EFSA opinion [123] stated that investigations in Germany indicated an increase in the occurrence of *C. purpurea* infections in the last 10 years. The opinion also mentioned that the increase seems to be associated with the

more extensive use of hybrid varieties of rye and perannial rye breeds and that infectivity may reach (in Germany) 40–50% of all investigated rye samples and may comprise also sclerotia from grass-contaminating harvested cereals. Separation of the ergots from grain and/or the use of fungicides could eliminate most of the risks. TDI values for these contaminants in the food chain are not available. Animal as well as clinical studies on some EAs enabling definition of NOAELs seem to be available, but for establishment of a TDI, data on long-term toxicity, reproductive and developmental toxicity, genotoxicity and carcinogenicity are also needed.

6 Alkaloids derived from nicotinic acid

Alkaloids derived from nicotinic acid are nicotine and myosmine.

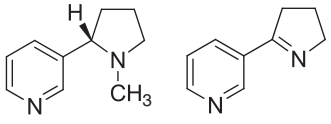
6.1 Alkaloids derived from nicotinic acid (nicotine, myosmine)

6.1.1 Alkaloids derived from nicotinic acid (nicotine, myosmine): chemical characteristics and dietary occurrence

Nicotine (Fig. 10) is mainly found in the tobacco plants *Nicotiana tabacum* and *N. rustica*. Tobacco leaves contain from 0.6 to 9% (6–90 g/kg) nicotine [143]. Nicotine is also found in a number of food plants, i.e. 2–7 μg/kg in some fresh fruits [144], 5–43 μg/kg of wet weight in tomatoes and various parts thereof and 16.8 μg/kg of wet weight in cauliflower [145–147]. Others have shown that green peppers and eggplant (aubergine) may contain up to 100 μg/kg [71] and the latter is richer in nicotine than any other edible plant [146]. Regular and decaffeinated black teas have a nicotine content ranging from non-detectable to 127.9 μg/kg of wet weight depending on the way of tea preparation [145].

Tomatoes, potatoes, aubergines and tea are the four major dietary sources of nicotine. The mean daily dietary intake of nicotine in Italy and Portugal was estimated at 1.4 μg/day [144]. This value is lower than in a study performed with non-smokers in the USA, where an average and MDI of nicotine was estimated at 8.8 μg/day and 99.9 μg/day, respectively [145].

Myosmine, 3-(1-pyrroline-2-yl)pyridine (Fig. 10) is another tobacco alkaloid found in many foods. Myosmine has been detected in peanuts (*Arachis hypogaea*) at 0.2–2.0 ng/g and in hazelnuts (*Corylus avellana*) at 0.7 ng/g



nicotine myosmine

Figure 10. Structures of nicotine and myosmine.

[148]. Other dietary sources of myosmine are rice, corn, wheat flour, millet, almonds, cocoa, cereals, popcorn, tomatoes, potatoes, carrot, pineapple, kiwi fruit, apple and milk [148, 149]. No myosmine was detectable in other vegetables and fruits such as lettuce, spinach, cucumber, onion, banana, tangerines and grapes [149]. Consumption of 250 g of peanuts or hazelnuts would translate to a myosmine uptake of 0.05–0.5 μg [148].

6.1.2 Alkaloids derived from nicotinic acid (nicotine, myosmine): possible adverse health effects and safety assessments

Nicotine acts on the nicotinic acetylcholine receptors. The diverse effects induced by nicotine upon smoking, e.g. increased heart rate and blood pressure, provoked cardiac contractility, decreased skin temperature, mobilized blood sugar, increased catecholamine levels in the blood, cardiovascular disease, atherosclerosis, pulmonary and neoplastic disorders, do not occur at the oral dose levels at which nicotine originating from food is consumed [2, 143].

Up to now, given the low acute toxicity of myosmine and the weak affinity of myosmine for nicotinic receptors, no attention has been directed at its safety assessment. This may change now that it has been discovered that myosmine has adverse effects of its own and occurs independently of nicotine in a great variety of staple foods, vegetables, fruits, nuts and dairy products and consequently in human milk, plasma and saliva [150]. Due to its imine structure, myosmine may be readily converted to carcinogenic substances such as the nitrosamine *N'*-nitrosonornicotine [149, 150]. Endogenous nitrosation of myosmine may lead to metabolites that have been shown in other studies to induce tumours in the upper intestinal tract (such as oesophageal adenocarcinoma), the oral cavity and the respiratory tract in rodents and humans [150–153].

6.1.3 Alkaloids derived from nicotinic acid (nicotine, myosmine): conclusions

A cigarette contains about 8–9 mg of nicotine, 1 mg of which is delivered to the smoker. This amount corresponds to the consumption of 10 kg of unprocessed aubergines or 20 kg of pureed tomatoes. Thus, the exposure to nicotine from food appears to be negligible compared to that from active smoking and poses no health concerns.

The recent discovery that myosmine and especially its metabolites are potential carcinogens may cast a different look on this compound. Myosmine occurs in many foods (hazelnuts, peanuts and products made of them) and may present a risk although estimation of this risk requires further characterisation of myosmine exposure via food, its bioavailability upon oral exposure and its bioactivation, genotoxicity and potential carcinogenicity.

7 Glycoalkaloids (GAs)

GAs, also called steroid alkaloids, are an important class of food-borne alkaloids.

7.1 GAs (steroid alkaloids)

7.1.1 GAs (steroid alkaloids): chemical characteristics and dietary occurrence

A GA consists of a C_{27}-steroidal nitrogen-containing alkaloid (aglycone, alkamine) and a sugar moiety, often a tri- or tetrasaccharide, attached to the 3-OH position. Synonyms for GAs are solanins, *Solanum* alkaloids, steroidal GAs or basic saponins [154]. Some examples of aglycones are solanidine, tomatidine and solasodine with the corresponding glycosides being α-solanine and α-chaconine (from potatoes), tomatine (from tomatoes), solasonine (from eggplant), respectively (Fig. 11).

GAs are part of the human food chain, since they occur in potatoes, tomatoes, eggplant and capsicum (bell peppers). These well-known food products of the Solanaceae family can contain high amounts of toxic GAs, including α-solanine, α-chaconine and solasonine. α-Solanine and α-chaconine are the major GAs (90–95%) in potatoes (*S. tuberosum*) of which α-chaconine is the more toxic one [155]. The ratio α-chaconine/α-solanine varies from 2:1 to 1:1 [156, 157]. Other potato GAs and aglycones are solanidine, α- and β-solamargines (Fig. 11), demissine, demissidine, leptines I and II, liptidine, leptinines I and II, leptinidine, leptimidine and tomatidenol. The average solanidine glycoside content of 521 samples of potato tubers was 73 mg/kg (range: 10–390 mg/kg) [158]. Lampitt et al. give an average of 25–100 mg/kg [159]. The glycosides are usually concentrated in a 1.5 mm layer under the skin and peeling thus removes 50–95% of the GAs [158]. With regard to processed potatoes, the highest content of GAs is found in fried peels (1390–1450 mg/kg), followed by chips (26.7–162 mg/kg), frozen baked potatoes (80.2–123 mg/kg), dehydrated potato flakes (14.9–22.8 mg/kg), frozen french fries (3.3–57.8 mg/kg) and the lowest levels are found in canned peeled whole potatoes (0.9–1.5 mg/kg) [157]. GAs are hardly affected by food processing (baking, cooking, frying) but their content is influenced by the type of cultivar and original total GA content, light, mechanical injury and storage [157, 158, 160].

7.1.2 GAs (steroid alkaloids): possible adverse health effects and safety assessments

GAs (α-solanine, α-chaconine, tomatine and solasonine) are less toxic than non-glycosylated alkaloids, because the sugar moiety hampers absorption, thereby reducing bioavailability. In vivo hydrolysis results in formation of the aglycones solanidine, tomatidine and solasodine and may occur either by hydrochloric acid in the stomach or enzymatically by bacterial glycosidases in the gastrointestinal tract [161]. Toxicokinetic studies in rat and hamsters reported the bioavailability of unmodified α-solanine to be only 1.6 and 3.2%, respectively [162]. Humans appear to be more sensitive than animals [155]. A study in human volunteers

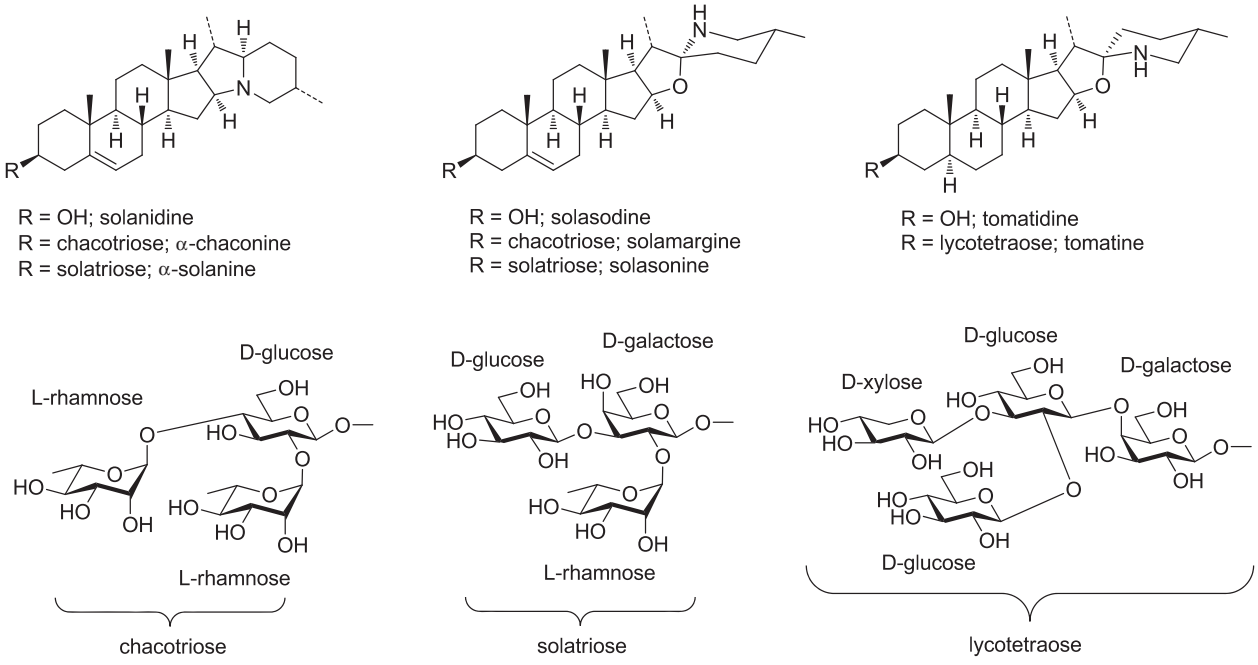


Figure 11. Structures of GAs.

reported α-solanine and α-chaconine in all blood samples collected from seven volunteers after a meal of potatoes with plasma concentrations up to about 7 and 14 ng/mL (μg/L), respectively [163]. Mensinga et al. reported similar figures in their volunteer study [164]. They demonstrated that none of the serum GA concentrations returned to baseline within 24 h post dose. α-Chaconine and α-solanine were shown to have half-lives of on average 44 and 21 h, respectively, implying that daily consumption may cause accumulation of GAs in the body [164].

α-Solanine and α-chaconine poisoning results in gastrointestinal disturbances when consumed at low doses, e.g. vomiting, diarrhoea and usually severe abdominal pain and, when consumed at high doses, also neurological disorders including drowsiness and apathy, confusion, weakness and visual disturbances [155, 160, 161]. The adverse effects include fever, rapid, weak pulse, low blood pressure and rapid respiration [155, 165]. McMillan and Thompson reported on an epidemic of potato poisoning among 78 school boys after having eaten a lunch with prepared potatoes containing 250–300 mg/kg GAs [166]. Seventeen of the boys were hospitalised. It has also been reported that potato GAs adversely affect intestinal permeability and aggravate inflammatory bowel disease [167]. They are potent irritants of the intestinal mucosa (lytic effect) and cholinesterase (acetyl- and butyrylcholinesterase) inhibitors [160, 168]. Based on studies in mice, potatoes containing more than 200 mg GAs/kg are considered to cause acute poisoning but little is known about long-term effects of repeated ingestion of small amounts of potato GAs [158, 167, 169]. This value of 200 mg GAs/kg being of concern with respect to acute poisoning is somewhat different from the results from a limited study in humans cited by JECFA indicating that the daily consumption of 200–300 g potato tubers containing approximately 24 mg of GAs/100 g (240 mg/kg), giving a daily intake of approximately 1 mg/kg bw/day, did not result in any signs of acute toxicity in humans (available at: http://www.inchem.org/documentsjec/fa/jecmono/v30je19.htm) [170]. JECFA however also refers to another study where intake of 1–1.5 kg cooked potatoes containing 24 mg GAs/100 g (240 mg/kg), equivalent to about 3.4–5.1 mg/kg bw, resulted in typical 'solanine' poisoning [170].

Initially, it was thought that GAs were teratogenic on the basis of a limited epidemiology [171]; however, subsequent epidemiological studies in man and developmental toxicity studies in laboratory animals did not confirm that GAs cause an increase in neural tube defects in humans [172–175].

In general, normal consumption of potatoes does not lead to adverse effects in humans. The maximum safe acute oral dose of total potato GAs has been estimated to be about 1 mg/kg bw, the acute toxic dose between 2 and 5 mg total GAs/kg bw, a dose between 3 and 6 mg total GAs/kg bw may be lethal [155, 158, 161, 176]. It is clear that a maximum safe dose of 1 mg/kg bw/day amounting to 60 mg/day for a 60 kg person and to 25 mg/day for a 25 kg child leaves only a small MOS compared to the acute toxic dose of 2–5 mg/kg bw/day amounting to 120–300 mg/day for a 60 kg person and 50–125 mg/day for a 25 kg child. Thus, consumption of a 500 g portion of potatoes containing 200 mg/kg of GAs could already cause acute toxic effects. Therefore, the GA level in potatoes for human consumption should preferably be lower than 100 mg/kg.

α-Tomatine is 20 times less toxic than the potato GAs [177]. Peruvians eat tomatoes with a very high α-tomatine content (500–5000 mg/kg of dry weight) without adverse effects and no poisoning in humans due to tomatoes has been reported. From this it can be concluded that the GAs in tomatoes are safer than those in potatoes [178].

The JECFA concluded that the large body of experience with the consumption of potatoes, frequently on a daily basis, indicates that normal GA levels (20–100 mg/kg) found in properly grown and handled tubers are not of concern [170].

7.1.3 GAs (steroid alkaloids): conclusions

In general, normal consumption of potatoes does not have adverse effects on humans. This is due to restrictions in the level of GAs, since potatoes destined for consumption in Europe and the USA contain 20–150 mg of total GAs/kg unpeeled tubers [155]. Control measures should be maintained, especially because potatoes that have been exposed to light, mechanical injury and started to green can have concentrations of 1000 mg/kg or more [179]. Other applications of potatoes in food such as fried potato skin, potato chips and baked jacked potatoes may result in products with GA levels that are higher than the levels found in peeled boiled potatoes. This, in combination with improper treatment or processing of these food items can lead to high intake of GAs for a longer period, and indicates a need for long-term toxicity studies and a more extended risk assessment.

8 Purine alkaloids

Caffeine, theobromine and theophylline belong to the group of the methylxanthine alkaloids derived from adenine/guanine. These are discussed below.

8.1 Methylxanthine alkaloids

8.1.1 Methylxanthines: chemical characteristics and dietary occurrence

Methylxanthines include caffeine, theobromine, 7-methylxanthine and theophylline (Fig. 12). Caffeine (1, 3, 7-trimethylxanthine) is the bitter-tasting alkaloid found in coffee, tea and cola. Coffee seeds contain 1–2% (10–20 g/kg) caffeine and traces of theophylline and theobromine.

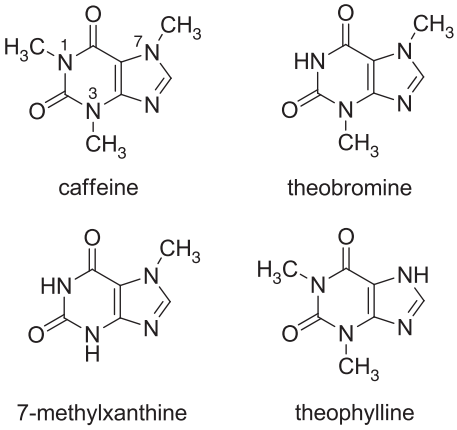


Figure 12. Structures of methylxanthines.

Brewed coffee contains 360 mg/L [180], instant coffee 118–316 mg/L and decaffeinated coffee less than 20 mg/L [180].

Tea contains 1–4% (10–40 g/kg) caffeine and small amounts (up to 0.05% (0.5 g/kg)) of both theophylline and theobromine [2]. The amount of methylxanthine alkaloids in teas depends on duration of brewing, the way of making tea (in Western countries mostly bags are used, in the Asian culture loose leaves) and how often one bag or the leaves are used.

Cola seeds contain up to 3% (30 g/kg) of caffeine, partly bound to tannins [2]. Caffeine containing drinks including cola drinks and energy drinks contain normally 65–250 mg caffeine/L [181]. Caffeine is a stimulant. It is added to some soft drinks as a flavouring ingredient and to increase the basal metabolic rate.

Cocoa seeds contain 35–50% (350–500 g/kg) oil (cocoa butter or theobroma oil), 1–4% (10–40 g/kg) theobromine and 0.2–0.5% (2–5 g/kg) caffeine, plus tannins and volatile oils. Theobromine is the major alkaloid found in cocoa plants, but due to fermentation and processing it is hardly found in different cocoa products [182]. According to another study, the highest amounts of theobromine are found in black and green tea [183]. Chocolate/cocoa drinks contain 21 mg of caffeine/L [180]. Theophylline can also be found in coffee, chocolate and cola beverages, but only in small quantities [184, 185].

8.1.2 Methylxanthine alkaloids: possible adverse health effects and safety assessments

Methylxanthines, and in particular caffeine, exert various effects on metabolic targets (e.g. satiety, thermogenesis and fat oxidation) [186]. The thermogenesis involves inhibiting the phosphodiesterase-induced intracellular degradation of cyclic adenosine monophosphate and antagonising adenosine receptors that have a negative effect on increased noradrenaline release [187, 188].

Caffeine is worldwide the most consumed psychoactive substance. It can cause changes in adult behaviour (mood, sleep pattern). Caffeine consumption at doses amounting to 50–200 mg/day causes a reduction in drowsiness and fatigue. Consuming amounts of methylxanthines of over 200 mg/day, especially caffeine, can provoke headache, nervousness, irritability, tremors, central convulsions, negative effects on premenstrual syndrome, fertility and pregnancy, and cardiovascular effects [183]. Above 400 mg/day caffeine may cause general toxicity like tremor, gastrointestinal problems, cardiovascular problems, including cardiac arrhythmias, and high blood pressure. Oral doses between 5 and 10 g of caffeine are lethal to man [189].

Some studies on the overall health risks of coffee or caffeine suggested the probability of cancer, including pancreatic, bladder, stomach and ovarian cancers, or leukemia, fibrocystic breast disease and gallbladder disease [190–192]. However, in epidemiological studies, no risks of coffee, including decaffeinated coffee and tea for colorectal cancer was established [193, 194]. Patients with a non-fatal first myocardial infarction taking at least five cups/day have been reported to be prone to increase the risk of a second incidence [195].

It has been concluded that pregnant women should limit coffee consumption to three cups/day to exclude any increased probability of spontaneous abortion or impaired fetal growth [196]. In a perspective cohort study with 1063 women, the course of pregnancy up to the 20th week has been recorded. Compared to pregnant women with no caffeine intake, the women who were exposed daily to 200 mg of caffeine were at increased risk of miscarriage (15 versus 12%) and the corresponding risk for pregnant women with a caffeine intake of more than 200 mg was considerably higher (25 versus 12%). This result was independent of the type of caffeine-containing preparation [197]. Results from a large prospective observational study indicated that maternal caffeine intake during pregnancy is associated with an increased risk of impaired foetal growth. The threshold at which this risk is significantly higher is not well characterised, but the data confirmed that the association of impaired foetal growth with caffeine is reduced for those consuming <100 mg/day [198]. Based on the new results on maternal caffeine intake during pregnancy and the risk of impaired foetal growth [198], the Food Standard Agency gave the advice to pregnant women to limit their daily caffeine intake, ideally keeping this below 200 mg/day [199].

In contrast to the other methylxanthines, the action of theobromine on the central nervous system is low [185]. The only data about adverse effects reported in humans upon intake of high doses of theobromine are nausea and anorexia [185].

Theophylline is medically used to treat asthma due to its bronchodilating effect. Theophylline toxicity symptoms range from seizures, tachycardia, nausea and vomiting, hypokalaemia, headache and tremors. Cardiac arrest and

arrhythmias, and hypotension were also frequently recorded in patients or healthy subjects taking theophylline [185]. Different authors report different values regarding the plasma levels of theophylline. In general, below 8–15 μg/mL theophylline causes no toxicity, between 9 and 20 μg/mL mild toxicity, above 25 μg/mL severe toxicity and above 55 μg/mL death [185].

According to Directive 2002/67/EC of the Commission of 18 July 2002 caffeine used as a flavouring in the production or preparation of a foodstuff must be mentioned by name in the list of ingredients on the label (Directive 2000/13/EC Article 3(1)(2)) immediately after the term 'flavouring'. When a beverage contains caffeine in excess of 150 mg/L, the message 'high caffeine content' must appear on the label followed by the caffeine content expressed in mg/100 mL. The EFSA, in assessing caffeine and theobromine as flavouring substances, stated that the compounds could not be evaluated because information on production volumes and normal and maximum use levels were lacking [200].

8.1.3 Methylxanthine alkaloids: conclusions

Based on the new results on maternal caffeine intake during pregnancy and the risk of impaired foetal growth, pregnant women should restrict their intake of caffeine. For other adults consuming moderate amounts of coffee (three to four cups/day providing 300–400 mg/day of caffeine), there is little evidence of health risks. Some groups (for example, people with cardiovascular diseases) may be more susceptible to the adverse effects of caffeine. Overall, toxicological risks of theobromine are negligible. Theophylline hardly occurs in the Western diet and thus does not constitute a health problem.

9 Concluding remarks

The present review describes the occurrence, concentration in specific foods and toxicological effects including the mode of action and existing safety assessments of alkaloids occurring in modern Western food. From this, it can be concluded that some alkaloids, such as 1,2 unsaturated PAs, are a reason for concern, because of their bioactivation to alkylating intermediates. In turn, these intermediates are able to react with cellular macromolcules causing cellular toxicity and upon their reaction with DNA causing genotoxicity and eventually carcinogenesis. Other groups of alkaloids, including QA, β-carboline, EAs and steroid alkaloids, are active without prior bioactivation and, in most cases, appear to act as neurotoxins by interacting with neurotransmitter systems.

For most compounds, regulatory agencies are aware of the problems encountered and have taken or are considering appropriate regulatory actions to protect the public when this is considered necessary. A general limitation for alkaloid-containing weed seeds and unground and uncrushed fruits to 3000 mg/kg of animal feed has been included in Directive 2002/32/EC, setting also limits of 1000 mg/kg for *Datura stramonium* seeds known to contain TAs and of 100 mg/kg for parts of *Crotalaria* spp. known to contain PAs [140]. For food, regulatory actions may vary from setting limits for the presence of a compound in foods and beverages (such as for PAs, β-carboline alkaloids and GAs), trying to define safe upper limits (such as for QAs, GAs, quinine and morphine), advising on a strategy aiming at restrictions in use (such as for PAs, QAs, EAs and β-carboline alkaloids), informing the public to be cautious and aware of possible adverse side effects (as for QAs, β-carboline alkaloids, quinine, EAs and caffeine), or taking specific plant varieties and/or their ingredients from the market (such as for certain botanicals containing PAs). For some alkaloids known to be present in Western foods, e.g. piperine, nicotine, theobromine and theophylline, and TAs, risks coming from the human food chain are considered to be negligible. Remarkably, for some alkaloids (such as QAs, EAs and myosmine) that are known constituents of possible concern in the modern food chain, TDIs have so far not been defined.

The authors have declared no conflict of interest.

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